**Original Article**

# Prevalence of Intestinal Protozoan Infection in Patients with Ulcerative Colitis (UC) in Isfahan, Iran

## Abstract

**Background:** Determination of the prevalence of intestinal protozoan infection is a fundamental step to set up an effective control program to improve the health status of society and to establish efficient strategies. Intestinal pathogen and even non-pathogen protozoa consider as major causes of disease in patients with gastrointestinal problems. The objective of this study is to determine the prevalence of intestinal protozoan infection in patients with ulcerative colitis (UC) in Isfahan, Iran. **Methods:** The descriptive cross-sectional study carried out from 2013 to 2018 in Isfahan, Iran. One thousand nine hundred and sixty-five samples of feces from patients with UC collected and each sample examined using direct wet mounting with normal saline and iodine and sedimentation tests such as formol-ethyl acetate concentration and trichrome-staining methods. **Results:** From 655 patients, 185 (28.2%) infected with *Giardia lamblia* followed by *Blastocystis hominis* (27.3%), *Endolimax nana* (14.4%), *Entamoeba coli* (11.5%), *Iodamoba butschlii* (4.7%), *Entamoeba histolytica* (1.4%), and *Chilomastix mesnili* (0.6%). **Conclusions:** This study revealed a high prevalence of infection with at least one or six non-pathogenic and pathogenic intestinal protozoa in UC patients in the Isfahan region. Intestinal protozoa are a challenging public health problem wherever health care is limited in the area. The emergence of UC in the world results in the need to study etiologic factors. In order to obtain further information about the etiology of disease, we investigated the prevalence of intestinal protozoan infection in patients with UC in Isfahan, Iran.

**Keywords:** *Blastocystis hominis, Giardia lamblia,* intestinal protozoa, Iran, ulcerative colitis

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## Introduction

Intestinal parasitic infections are still one of the most important health problems in some developing countries.[1] About 3.5 billion people around the world infected with intestinal parasites and over 450 million people are ill due to intestinal parasite infections.[2] These infections are often asymptomatic or show mild symptoms but severing or chronic complications could occur.[3] Two major types of parasites, protozoa and helminths, infect the gastrointestinal tract (GI) and can cause wide ranges of gastrointestinal complaints from mild diarrhea to serious complications. Important intestinal protozoa, *Giardia lamblia* and *Blastocystis hominis* are health challenges of both developed and developing countries.[4,5] Recently, *B. hominis* found to be a specific pathogen in abdominal pain, diarrhea, anorexia, and tenesmus complications. In 1991, *B. hominis* seen in stool examination of a

man with ulcerative colitis (UC), after this observation, he treated with metronidazole successfully.[6]

UC is an idiopathic, chronic inflammatory disease of the colon. The exact cause of UC is unknown, but many possible factors such as genetics, nutritional status, stress, immune system dysfunction, changes in the normal gut microbial and bacterial, viral, fungal, and parasitic agents play a role. UC is considered as one of the common causes of chronic gastrointestinal disease in the world. At first, regarded as a Western lifestyle disease, UC is increasing in many parts of the world.[7-9] The reason for this growing trend has not been established.[10,11] UC involves the colon and rectum and mucosa, manifesting as continuous areas of inflammation and ulceration.[12] UC often presents with abdominal pain and diarrhea. The clinical manifestation of UC disease is not very different from symptoms of intestinal infection caused by parasites.[13] Despite its self-limited character, this infection leads to chronic,

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relapsing disease in susceptible patients.[14] Patients with severe UC should be hospitalized and corticosteroids must be prescribed. If corticosteroid has not been effective, surgical intervention must be carried out.[15,16] Patients with UC have an increased risk of colon cancer. The annual incidence of UC is between nine and 12 cases per 100,000 persons in the United States.[4] UC is a kind of disease with a complicated interaction between genetic, immune, and environmental factors. The etiology of UC is not well known but it presumed that recent infection with bacterial, viral, or parasitic agents could trigger the risk of subsequent development of UC. It is suggested that intestinal infection probably leads to changes in gut flora, triggering the onset of chronic inflammatory and UC complications.[17] Although, active UC associated with increased detection rates of parasites in the stool,[13] recent studies do not confirm the possible role of microorganisms like parasites in exacerbation of UC.[17] The association of parasitic infection with UC disease evoked much interest since some investigations found parasitic infection in the fecal exam of patients with UC.[18] Hence, for more information about this correlation, more studies need to carry out and we intend to investigate the possible role of intestinal protozoa in UC.

## Methods

In the current cross-sectional, epidemiological, and descriptive study, three fresh feces samples of 655 patients with UC who referred to Isfahan health care centers from 2013 to 2018 were collected. An informed consent form signed by all the participants in this study (in cases under 18-year-old, it was signed by their parents). The diagnosis of UC carried out based on clinical, endoscopic, radiological, and histological parameters. All the fresh feces samples transferred to the laboratory, immediately and a stool exam carried out promptly for each sample. The whole study approved by the relevant ethics committee of the Isfahan University of Medical Science, Iran, (ethical code: 291311).

### Fecal examination

First, macroscopic observation of each sample such as consistency and other visual features carried out. Then, a microscopic examination performed based on National Committee for Clinical Laboratory Standards (NCCLS) as follows: direct wet mounting with normal saline and iodine and sedimentation technique like the formol-ethyl acetate concentration and trichrome-staining method. The samples examined for intestinal protozoa under a light microscope.

### Data analysis

All the obtained data analyzed using SPSS version 21.0 software. Descriptive statistics utilized to assess the prevalence of intestinal protozoan infection. The Chi-square test was used to show possible differences. The significance level was 0.05.

## Results

Samples of 655 patients with UC collected. Out of 655 patients, 312 (47.6%) were male and 343 (52.4%) were female. This shows the higher prevalence of UC in females but generally no statistically significant difference seen between different sexes ($P = 0.7$). The majority of cases, that is, 234 (35.7%), were of 0–10 years old. As for other age groups, 26.3%, 17.6%, 15.1%, 4.6%, 0.6% were of 11–20, 21–30, 31–40, 41–50, and 51–60 years old, respectively. The patients between 61 and 70 years old were the lowest among others, 0.2%. Four hundred and five (61.8%) of cases were single and others were married. This result showed the higher prevalence of UC in singles but no significant relationship observed between marital status and the prevalence rate of disease ($P = 0.06$).

Frequencies of intestinal protozoan infection among 655 cases were as follows: 185 (28.2%) infected with *G. lamblia* which had the highest prevalence among the patients, followed by *B. hominis* (27.3%), *Endolimax nana* (14.4%), *Entamoeba coli* (11.5%), *Iodamoba butschlii* (4.7%), *Entamoeba histolytica/dispar* (1.4%), and *Chilomastix mesnili* (0.6%).

The prevalence of intestinal infections in the different age groups of patients with UC in Isfahan, Iran from 2013 to 2018 presented in Figure 1. *G. lamblia* was the most prevalent protozoa in the 0–10-year old patients (37.2%), while in age groups of 11–40 years old; *B. hominis* was the most prevalent one, but no statistically significant difference seen between the age and type of intestinal protozoa.

In the age group of 51–60 years old, no infection with *B. hominis* observed. No protozoan infection was seen in the 61–70 years old group. The prevalence of *E. histolytica/dispar* in patients between 11 and 20 years old was the highest among other age groups (2.3%).

The prevalence of intestinal protozoan infections in males and females with UC in Isfahan, Iran from 2013 to 2018 shown in Figure 2. *G. lamblia* was the most prevalent
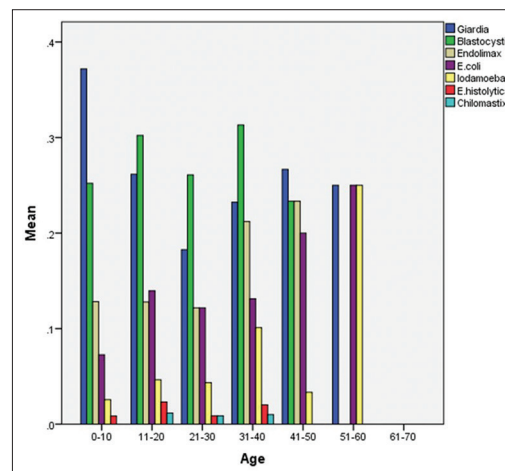


**Figure 1: Prevalence of intestinal protozoan infections in different age groups of patients with ulcerative colitis in Isfahan, Iran from 2013 to 2018**
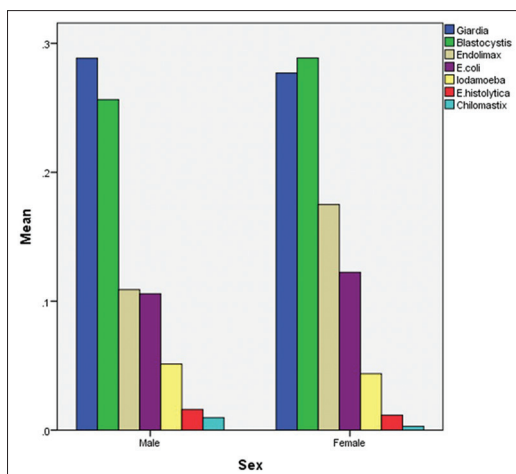
Figure 2: Prevalence of intestinal protozoan infections in males and females with ulcerative colitis in Isfahan, Iran from 2013 to 2018

parasite in men while *B. hominis* infection occurred in women more than men. There was no statistically significant difference between sex and intestinal protozoa except for *E. nana*, in the case of *E. nana* the prevalence rate of protozoan infection in males and females showed a meaningful difference, ($P < 0.05$). The prevalence rate of infection with *E. nana* in females was greater than men: 17.5% of women and 10.9% of men infected with *E. nana*.

## Discussion

UC is one of the usual reasons for gastrointestinal disease in the world. As shown in multiple studies, the incidence and prevalence of UC increased in Asia which shows the importance of the disease.[7,19,20] In other parts of the world, such as Mexico, the mean annual number of UC cases grew from 28.8% in 1987–1996 to 76.1% in 1997–2006.[21] The proof for this growth has not been clarified but is probably related to environmental factors, including developed hygiene and alternation of diet.[9] Intestinal parasites are of serious problems all over the world.[22] Hence, in the current study, the prevalence of intestinal protozoan infection in UC patients investigated to signify the possible role of protozoa in UC disease and to determine whether there was any correlation between intestinal protozoan infection and UC disease.

Among 655 patients with UC, 52.4% were female patients which in contrast with some studies.[20,23] Totally, there appears to be no gender predominance in UC.[24] Most of the patients with UC in current study were under 40 years old. This is in line with the findings of several studies.[7,19,20] Also, several investigations stated that the peak age of disease is between ages 30 years and 40 years.[24,25] In current study, the prevalence of protozoan infection in every patient according to their age, investigated. The most prevalent parasite in UC patients under 10 years old was *G. lamblia*. The result confirms previous studies that found that children are more at risk

of infection with *G. lamblia* than adults, most probably due to the lack of natural or acquired immunity, direct and fecal-oral transmission of the parasite, low personal hygiene and eating of unwashed fruits and vegetables.[26-28] In age groups of 11–20, 21–30, and 31–40, *B. hominis* was seen more than *G. lamblia*. *B. hominis* presumably is the most widespread enteroparasitic organism in all over the world, with more than 50% prevalence rate.[29,30] Although, *B. hominis* known as a nonpathogenic agent, several reports of *B. hominis* infections were seen in literature; therefore, it should be noticed as a pathogen especially in patients with UC.[6,31-33] Meanwhile, no infection with *B. hominis* seen in the age group of 51–60 years old. No protozoan infection was seen in 61–70 years old ones maybe because they less exposed to infection with parasites. The reason for not observing infection in age groups of 51–60 years old and 61–70 years old may be due to their less exposure to infection sources and also, long time involvement of these patients with UC cause prolonged treatment with metronidazole which can prevent parasitic infection, as well. Also, the low rate of protozoan infections in elder patients shows the success of health care programs.[34] The prevalence rate of *E. histolytica/dispar* in patients in the age group of 11–20 years old was higher than the other age groups of the current study but it was not statistically significant. Since *E. histolytica/dispar* is not endemic in Isfahan and belongs to the most frequent protozoan parasites causing travelers' diarrhea, traveling history of patients should be considered.[35,36]

*G. lamblia* was more prevalent in men than women. This group is more likely to contact with *G. lamblia* and current finding is similar to previous study.[37] In current survey, the most prevalent infection in women was *B. hominis* followed by *G. lamblia*. The trend of prevalence of other different protozoa was similar in both men and women. The most prevalent protozoa in UC patients were *G. lamblia* and *B. hominis*, perhaps because most of the patients in the current study were under twenty years old and *G. lamblia* occur more in the age group under twenty years old.

In conclusion, in the current study, three feces samples collected from each patient, hence, obtained results are more acceptable compared with other studies performed by only one feces sample. The current study showed a relatively high prevalence of protozoan infection in UC patients, therefore, early detection and rapid treatment of protozoan infections can improve the clinical course of patients with UC. Clinicians should be informed about the possible role of protozoan infection in UC. Enhancing awareness and improving the knowledge of gastroenterologists and clinicians about opportunistic infections are important elements to optimize patient outcomes through the development of preventive or early diagnostic strategies. Also, it is necessary to develop instructions for laboratory staffs aimed at improving the diagnosis of both pathogen and non-pathogen protozoa.

Although the exact role of parasites in the pathogenesis of UC is unknown, it seems essential that patients with UC undergo routine parasitological investigations. More studies on different aspects of UC and the possible role of parasitic infection on it should be carried out in the future. A study with a larger sample size and comparison with case group should be noticed for future works. subtypes of *B. hominis* and genotypes of *G. lamblia* in UC patients and control groups should be determined in the next surveys. Finally, because the majority of protozoan infections in this study are among water-borne infections, results emphasize the importance of screening of the water to prevent possible of the spread of parasitic protozoan.

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## Conflict of interest

There are no conflicts of interest.

## References

1. Anthony RM, Rutitzky LI, Urban JF Jr, Stadecker MJ, Gause WC. Protective immune mechanisms in helminth infection. Nat Rev Immunol 2007;7:975-87.
2. Pestehchian N, Nazari M, Haghighi A, Salehi M, Yosefi HA, Khosravi N. Prevalence of intestinal parasitic infection among inhabitants and tribes of Chelgerd, Iran, 2008-2009. J Clin Diagn Res 2015;9:LC01-4.
3. Bahmani P, Maleki A, Sadeghi S, Shahmoradi B, Ghahremani E. Prevalence of intestinal protozoa infections and associated risk factors among schoolchildren in Sanandaj City, Iran. Iran J Parasitol 2017;12:108-16.
4. Ordas I, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative colitis. Lancet (London, England) 2012;380:1606-19.
5. Hechenbleikner EM, McQuade JA. Parasitic colitis. Clin Colon Rectal Surg 2015;28:79-86.
6. Cekin AH, Cekin Y, Adakan Y, Tasdemir E, Koclar FG, Yolcular BO. Blastocystosis in patients with gastrointestinal symptoms: A case-control study. BMC Gastroenterol 2012;12:122.
7. Wei SC, Lin MH, Tung CC, Weng MT, Kuo JS, Shieh MJ, *et al*. A nationwide population-based study of the inflammatory bowel diseases between 1998 and 2008 in Taiwan. BMC Gastroenterol 2013;13:166.
8. Ooi CJ, Fock KM, Makharia GK, Goh KL, Ling KL, Hilmi I, *et al*. The Asia-Pacific consensus on ulcerative colitis. J Gastroenterol Hepatol 2010;25:453-68.
9. Asakura K, Nishiwaki Y, Inoue N, Hibi T, Watanabe M, Takebayashi T. Prevalence of ulcerative colitis and Crohn's disease in Japan. J Gastroenterol 2009;44:659-65.
10. Yao T, Matsui T, Hiwatashi N. Crohn's disease in Japan: Diagnostic criteria and epidemiology. Dis Colon Rectum 2000;43(10 Suppl):S85-93.
11. Zargar A, Gooraji SA, Keshavarzi B, Aghamohammadi AA. Effect of irritable bowel syndrome on sleep quality and quality of life of inflammatory bowel disease in clinical remission. Int J Prev Med 2019;10:10.
12. Colitis-Pathophysiology U. Inflammatory bowel disease part I: Ulcerative colitis–pathophysiology and conventional and alternative treatment options. Altern Med Rev 2003;8:247-83.
13. Banerjee D, Deb R, Dar L, Mirdha BR, Pati SK, Thareja S, *et al*. High frequency of parasitic and viral stool pathogens in patients with active ulcerative colitis: Report from a tropical country. Scand J Gastroenterol 2009;44:325-31.
14. Yamamoto-Furusho JK, Torijano-Carrera E. Intestinal protozoa infections among patients with ulcerative colitis: Prevalence and impact on clinical disease course. Digestion 2010;82:18-23.
15. Adams SM, Bornemann PH. Ulcerative colitis. Am Fam Physician 2013;87:699-705.
16. Baghaei A, Emami MH, Adibi P, Tavakkoli H, Daghaghzadeh H, Tamizifar B, *et al*. Inflammatory bowel disease registry and monitoring: Feasibility study and application (Isfahan Inflammatory Bowel Disease Surveillance Project). Int J Prev Med 2019;10:190.
17. Iyer VH, Augustine J, Pulimood AB, Ajjampur SS, Ramakrishna BS. Correlation between coinfection with parasites, cytomegalovirus, and *Clostridium difficile* and disease severity in patients with ulcerative colitis. Indian J Gastroenterol 2013;32:115-8.
18. Weinstock JV, Elliott DE. Helminths and the IBD hygiene hypothesis. Inflamm Bowel Dis 2008;15:128-33.
19. Quezada SM, Cross RK. Association of age at diagnosis and ulcerative colitis phenotype. Dig Dis Sci 2012;57:402-7.
20. Shivashankar R, Tremaine WJ, Harmsen WS, Loftus EV. Incidence and prevalence of Crohn's disease and ulcerative colitis in Olmsted County, Minnesota from 1970 through 2010. Clin Gastroenterol Hepatol 2017;15:857-63.
21. Yamamoto-Furusho JK. Clinical epidemiology of ulcerative colitis in Mexico: A single hospital-based study in a 20-year period (1987-2006). J Clin Gastroenterol 2009;43:221-4.
22. Vahedi M, Gohardehi S, Sharif M, Daryani A. Prevalence of parasites in patients with gastroenteritis at East of Mazandaran Province, Northern Iran. Trop Biomed 2012;29:568-74.
23. Krogsgaard LR, Engsbro AL, Stensvold CR, Nielsen HV, Bytzer P. The prevalence of intestinal parasites is not greater among individuals with irritable bowel syndrome: A population-based case-control study. Clin Gastroenterol Hepatol 2015;13:507-13.e2.
24. Cosnes J, Gower-Rousseau C, Seksik P, Cortot A. Epidemiology and natural history of inflammatory bowel diseases. Gastroenterology 2011;140:1785-94.e4.
25. Shapiro JM, Zoega H, Shah SA, Bright RM, Mallette M, Moniz H, *et al*. Incidence of Crohn's disease and ulcerative colitis in Rhode Island: Report from the Ocean State Crohn's and Colitis Area Registry. Inflamm Bowel Dis 2016;22:1456-61.
26. Sánchez A, Munoz M, Gómez N, Tabares J, Segura L, Salazar Á, *et al*. Molecular epidemiology of *Giardia*, *Blastocystis* and *Cryptosporidium* among indigenous children from the Colombian Amazon Basin. Front Microbiol 2017;8:248.
27. Hanevik K, Dizdar V, Langeland N, Hausken T. Development of functional gastrointestinal disorders after *Giardia lamblia* infection. BMC Gastroenterol 2009;9:27.
28. Mohammed Mahdy A, Lim Y, Surin J, Wan KL, Al-Mekhlafi MH. Risk factors for endemic giardiasis: Highlighting the possible association of contaminated water and food. Trans R Soc Trop Med Hyg 2008;102:465-70.

29. Petersen AM, Stensvold CR, Mirsepasi H, Engberg J, Friis-Møller A, Porsbo LJ, *et al*. Active ulcerative colitis associated with low prevalence of Blastocystis and *Dientamoeba fragilis* infection. Scand J Gastroenterol 2013;48:638-9.

30. Kuo HY, Chiang DH, Wang CC, Chen TL, Fung CP, Lin CP, *et al*. Clinical significance of Blastocystis hominis: Experience from a medical center in northern Taiwan. J Microbiol Immunol Infect 2008;41:222-6.

31. Jeddy T, Farrington G. Blastocystis hominis complicating ulcerative colitis. J R Soc Med 1991;84:623.

32. Mirjalali H, Abbasi MR, Naderi N, Hasani Z, Mirsamadi ES, Stensvold CR, *et al*. Distribution and phylogenetic analysis of Blastocystis sp. subtypes isolated from IBD patients and healthy individuals in Iran. Eur J Clin Microbiol Infect Dis 2017;36:2335-42.

33. Rossen NG, Bart A, Verhaar N, van Nood E, Kootte R, de Groot PF, *et al*. Low prevalence of Blastocystis sp. in active ulcerative colitis patients. Eur J Clin Microbiol Infect Dis 2015;34:1039-44.

34. Nitzan O, Elias M, Peretz A, Saliba W. Role of antibiotics for treatment of inflammatory bowel disease. World J Gastroenterol 2016;22:1078-87.

35. Pestehchian N, Nazary M, Haghighi A, Salehi M, Yosefi H. Frequency of Entamoeba histolytica and Entamoeba dispar prevalence among patients with gastrointestinal complaints in Chelgerd city, southwest of Iran. J Res Med Sci 2011;16:1436-40.

36. Herbinger K-H, Fleischmann E, Weber C, Perona P, Löscher T, Bretzel G. Epidemiological, clinical, and diagnostic data on intestinal infections with Entamoeba histolytica and Entamoeba dispar among returning travelers. Infection 2011;39:527-35.

37. Snel SJ, Baker MG, Venugopal K. The epidemiology of giardiasis in New Zealand, 1997-2006. N Z Med J 2009;122:62-75.